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Feature Review

Nociceptor Sensory Neuron–Immune Interactions in Pain and Inflammation

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Nociceptor sensory neurons protect organisms from danger by eliciting pain and driving avoidance. Pain also accompanies many types of inflammation and injury. It is increasingly clear that active crosstalk occurs between nociceptor neurons and the immune system to regulate pain, host defense, and inflammatory diseases. Immune cells at peripheral nerve terminals and within the spinal cord release mediators that modulate mechanical and thermal sensitivity. In turn, nociceptor neurons release neuropeptides and neurotransmitters from nerve terminals that regulate vascular, innate, and adaptive immune cell responses. Therefore, the dialog between nociceptor neurons and the immune system is a fundamental aspect of inflammation, both acute and chronic. A better understanding of these interactions could produce approaches to treat chronic pain and inflammatory diseases.

Trends

A bidirectional crosstalk between nociceptor sensory neurons and immune cells actively regulates pain and inflammation.

Immune cells release lipids, cytokines, and growth factors that have a key role in sensitizing nociceptor sensory neurons by acting in peripheral tissues and the spinal cord to produce neuronal plasticity and chronic pain.

Nociceptor neurons release neuropeptides that drive changes in the vasculature, lymphatics, and polarization of innate and adaptive immune cell function.

Nociceptor neurons modulate host defenses against bacterial and fungal pathogens, and, in some cases, neural activity benefits the host, while in others, it benefits the pathogen.

Interactions between nociceptor neurons and immune cells contribute to pathology in chronic inflammatory diseases, including rheumatoid arthritis, psoriasis, asthmatic lung disease, and colitis.

Neuronal Pathways of Pain Sensation

Pain is one of four cardinal signs of inflammation defined by Celsus during the 1st century AD (*De Medicina*). Nociceptors are a specialized subset of sensory neurons that mediate pain and densely innervate peripheral tissues, including the skin, joints, respiratory, and gastrointestinal tract. Various subsets of nociceptors exist, and can respond to mechanical, chemical, or thermal noxious stimuli (Box 1). Nociceptor nerve terminals express ligand-gated and voltage-gated ion channels, including TRPV1, TRPA1, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$, which are key molecular transducers of these noxious stimuli (Box 2). Given the ability of the nervous system to propagate signals within milliseconds, nociceptors are ideally positioned to be the first responders to pathogens and tissue injury. While pain is critical to induce behavioral changes that lead to avoidance of noxious stimuli, it is also increasingly clear that pain sensation is closely linked to molecular and cellular interactions between the nervous and immune systems. Immune cells release mediators that modulate nociceptor neuron activity and pain sensitivity. Nociceptors in turn release neuropeptides and neurotransmitters that act on innate and adaptive immune cells to modulate their function. Thus, neural signaling can define the pattern of immune responses and, consequently, contribute to the development of local and systemic inflammatory diseases. In this review, we discuss recent advances in understanding this bidirectional neuroimmune crosstalk in pain and inflammation.

Modulation of Pain Sensitivity by Immune Cells

The immune system has a critical role in pain by releasing molecular mediators that sensitize nociceptor neurons. Tissue injury and inflammation are intimately coupled to increases in pain sensation. Nociceptor peripheral nerve terminals have receptors and ion channels that detect molecular mediators released during inflammation (Figure 1). Upon activation, action potentials

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Box 1. Nociceptive Neuron Subsets and Pain Sensitization

Nociceptor neurons show remarkable diversity, with various cellular subsets mediating heat, cold, and mechanical pain [103]. Specific coupling of nociceptor subtypes with distinct immune cell types at the molecular level could be a mechanism by which neuroimmune signaling is finely tuned. Nociceptor neuron subtypes innervate different epithelial layers in the skin, lung, and gastrointestinal tract, allowing them to interface with environmental stimuli. C-fiber nociceptor neurons are nonmyelinated, slow-conducting neurons that are mostly capsaicin sensitive and often mediate thermal pain sensitivity. Aβ and Aδ nociceptor neurons are faster-conducting, myelinated neurons, often mediating mechanosensation and mechanical pain sensitivity. However, these classic groupings are broad and overly simplistic. Recent work has shown that nociceptors are highly diverse with distinct molecular expression patterns of ion channels, growth factor receptors, G-protein-coupled receptors, and neuropeptides. Therefore, the same cell may be able to respond to multiple sensory stimuli and mediate distinct functional outcomes.

Pain sensitization is defined by the International Association for the Study of Pain (IASP) as an increased responsiveness of nociceptor neurons to their normal or subthreshold afferent input. Pain sensitization can be further categorized as hyperalgesia or allodynia. Hyperalgesia is increased pain due to a normally noxious stimuli, whereas allodynia is a painful response to normally innocuous mechanical or thermal stimuli.

Pain sensitization is mediated by multiple mechanisms at both the biophysical and transcriptional levels. Inflammatory stimuli,including cytokines, can induce phosphorylation of ligand-gated channels (e.g., TRPV1 or TRPA1) or modification of voltage-gated sodium channels (e.g., $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$), producing changes in membrane properties, increased action potential firing, and heightened sensitivity to thermal or mechanical stimuli. Ligand-gated G-protein-coupled receptors are often coupled with TRP channel signaling. For example, bradykinin is released during inflammation to activate the bradykinin receptor on nociceptors, inducing phospholipase C and protein kinase A signaling, which potentiate TRPA1 opening and pain signaling. Inflammation and injury can also lead to changes in the transcriptional profiles of DRG sensory neurons with upregulation of TRP channels or other noxious molecular transducers, allowing previously unresponsive neurons to gain the ability to respond to noxious stimuli. Furthermore, inflammatory responses can act through growth factor regulation to modulate both the quantity and quality of tissue innervation by nociceptor nerve endings. Therefore, the mechanisms of pain sensitization are complex and involve changes in nociceptor neurons at both the molecular and cellular level.

Box 2. Nociceptive Ion Channels as Molecular Transducers of Pain

$Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are voltage-gated sodium channels enriched in nociceptor neurons compared with other neuronal subtypes [104]. These channels shape action potential generation and are critical for nociceptor neuron depolarization, thus mediating the initiation of pain signaling. $Na_v1.7$ loss-of-function mutations have been linked to an inability to feel pain in humans [104]. $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ gain-of-function mutations have been linked to increased pain in inherited erythromelalgia and painful neuropathy [104]. Inflammatory signaling pathways in nociceptors can lead to phosphorylation or modification of cytoplasmic residues in $Na_v1.7$, $Na_v1.8$, or $Na_v1.9$, which induce more-ready action potential generation and pain sensitivity [105].

Transient receptor potential (TRP) ion channels, a protein family comprising 30 distinct subtypes in mammals are key mediators of thermal and mechanical sensation [106]. TRPV1 is the founding member of a group of TRP channels gated by temperature and is critical for the induction of heat pain hypersensitivity. It is also activated by capsaicin, the pungent ingredient in chili peppers. By contrast, TRPM8 is gated by cold temperatures and mediates cold pain hypersensitivity. TRPV1 and TRPM8 are expressed by mostly distinct neuronal subsets in adult animals.

TRPA1 is another nociceptive ion channel that is thought to have a role in both chemical and mechanical pain sensitivity. TRPA1 was first identified to mediate noxious responses to allyl isothiocyanates (from mustard oils) and allicin (garlic). These electrophilic reactive chemicals covalently modify intracellular cysteine residues of TRPA1, which leads to its gating and subsequent pain production. Mechanosensation in DRG A-fiber neurons and other cell types is mediated by the newly identified ion channel Piezo2 [107]. It remains to be determined whether TRPA1 and Piezo2 synergize in mechanical hypersensitivity and pain.

are transduced to nociceptor cell bodies within the dorsal root ganglia (DRG), and relayed to the spinal cord and brain to be processed as pain. During inflammation, the threshold for nociceptor neurons to fire action potentials is reduced, leading to pain sensitivity or 'hyperalgesia'. Chronic pain accompanies inflammatory conditions, including rheumatoid arthritis and inflammatory bowel disease. Recent studies have aimed to define the specific immune cells and mediators involved in chronic pain. Pain tends to reduce with resolution of the tissue immune response, highlighting the importance of the immune system in neuronal sensitization. A deeper

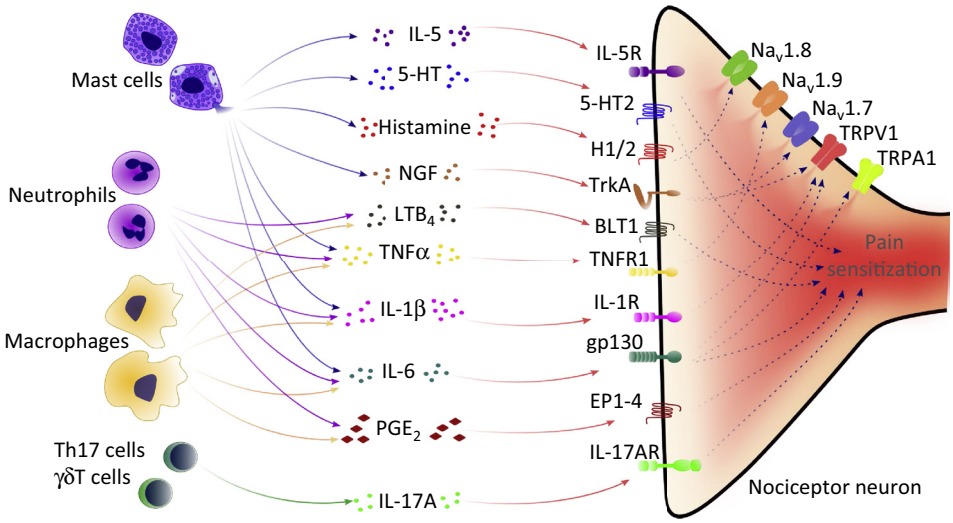

Figure 1. Immune Cells Release Mediators that Produce Peripheral Sensitization of Nociceptor Sensory Neurons and Pain. During inflammation, tissue-resident and recruited immune cells secrete molecular mediators that act on the peripheral nerve terminals of nociceptor neurons to produce pain sensitization. In these neurons, specific cytokine, lipid, and growth factor receptor intracellular signaling pathways lead to phosphorylation and/or gating of ion channels $Na_v1.7$, $Na_v1.8$, $Na_v1.9$, TRPV1, and TRPA1, leading to increased action potential generation and pain sensitivity. Upon degranulation, mast cells release Interleukin 5 (IL-5), serotonin (5-HT), histamine, and nerve growth factor (NGF) that act on IL-5R, 5-HT2, histamine receptor 2 (H2), TrkA, and nociceptor neurons, respectively, to produce pain sensitization. Nociceptor neurons are also sensitized by tumor necrosis factor alpha (TNFα), IL-1β, and IL-6 produced by mast cells, macrophages, and neutrophils. TNFα receptor 1 (TNFR1) activation leads to phosphorylation of $Na_v1.9$ channels. Activation of IL-1 receptor 1 (IL-1R1) increases TRPV1 expression by nociceptors, while IL-6 binds gp130 on nociceptors and this increases expression of both TRPV1 and TRPA1, enhancing responsiveness to heat and reactive chemicals. Prostaglandin E_2 (PGE_2) released by macrophages and other innate immune cells also sensitizes nociceptor neurons through PGE_2 receptors 1–4 (EP1–4). Th17 cells and γδT cells can also sensitize nociceptor neurons through IL-17A release and neuronal IL-17RA signaling.

understanding of how neuroimmune mechanisms produce neuronal sensitization could lead to treatments for chronic pain.

Immune Regulation of Peripheral Pain Sensitization

Nociceptor neurons express receptors for immune cell-derived cytokines, lipids, proteases, and growth factors (Figure 1). Upon activation, these receptors mediate signaling cascades that modify the gating properties of ion channels, including TRPV1, TRPA1, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$, through phosphorylation or other mechanisms, leading to increased neuronal firing (recently reviewed in [1]). Emerging studies have begun to elucidate the role of specific immune mediators in mediating pain sensitivity in different disease contexts. In mouse models of carrageenan-induced inflammatory pain [2] and neuropathic pain [3], neutrophils migrate to tissues where they sustain pain through the production of cytokines and prostaglandin E_2 (PGE_2). In incisional wound injury, non-neutrophilic $CD11b^+$ myeloid cells (potentially macrophages) are responsible for pain sensitization [4]. Mast cells also have key roles in sensitizing nociceptors. Electron microscopy indicates the close association of nociceptor nerve terminals with mast cells in mucosal tissues. Upon activation, mast cells degranulate and release cytokines (IL-5, TNFα, IL-6, and IL-1β), 5-HT, histamine, and nerve growth factor (NGF), which act through receptors on nociceptors, leading to pain sensitization [5–7]. In addition to their contribution to pain during acute inflammation [8], mast cells accumulate in chronic inflammatory conditions and contribute to the chronicity of pain [9]. Macrophages are sentinel myeloid cells present throughout the body, and monocytes are blood-borne myeloid cells prominently recruited to

inflammatory sites during tissue injury. A role for macrophages and monocytes in chronic painful disease conditions has been extensively demonstrated [10–14]. These cells produce many inflammatory cytokines, growth factors, and lipids that can act directly on nociceptor neurons to increase pain (Figure 1). T cells also have a role in neuropathic pain by releasing IL-17A and IFN-γ, which can act at nerve terminals to sensitize nociceptors [15].

In addition to neuroimmune interactions that occur at the site of injury, studies suggest that immune cells also interact with the cell bodies (somas) of nociceptor neurons within DRGs to produce pain. The soma represents an area that controls neuroplasticity and long-term sensitization through protein synthesis. A small number of innate and adaptive immune cells reside in DRGs and there is evidence that their numbers increase in chronic pain conditions. In chemotherapy- and sciatic nerve ligation-induced neuropathic pain, there are increases in the number of macrophages, monocytes, neutrophils, and T cells in the DRG [15,16]. T cells also release leukocyte elastase in the DRG to contribute to pain after nerve injury [17]. The role of activated mast cells in the DRG has also been related to pain in sickle cell disease [18]. Despite the work presented above, targeted analysis of how specific innate and adaptive immune cell types mediate pain sensitization is lacking in multiple disease states. However, recent work is beginning to define the critical role of immune mediators and their activation of neuronal signaling in chronic neuropathic and inflammatory pain. We summarize below some major classes of immune mediator that have been found to directly mediate nociceptor neuron sensitization and activation.

Lipid Mediators in Pain

Nonsteroidal anti-inflammatory drugs (NSAIDs) are potentially the most widely used pharmacological inhibitors of inflammatory pain. Their main mechanism of action is the inhibition of cyclooxygenases (COX), which produce prostanoids (prostaglandins, prostacyclins, and thromboxanes). PGE_2 is a potent booster of inflammatory pain. It activates neuronal EP1–EP4 receptors and sensitizes nociceptor neurons to other painful stimuli. PGE_2 acts as a sensitizer of nociceptor activity by acting on proximal ion channels, rather than as a direct activator of nociceptive neurons, an essential finding in understanding the analgesic effect of NSAIDs [19]. On a longer timescale, PGE_2 also induces persistent hyperalgesia via PKA and PKC-mediated activation of NF-κB in DRG neurons [20]. Other than prostaglandins, it is now clear that many classes of proinflammatory and anti-inflammatory lipid are involved in the activation and silencing of nociceptor activity (Figure 1). Lysophosphatidic acid and sphingosine-1-phosphate, for example, are produced during inflammation and act directly on nociceptor neurons to increase TRPV1 activity [21,22]. Leukotriene B_4 (LTB_4) injection induces hyperalgesia in humans [23], activating C-fibers and Aδ-fibers [24]. A subset of $TRPV1^+$ DRG neurons expresses BLT1, the receptor for LTB_4, which mediates calcium flux in response to ligand activation [25]. Recent work also highlights a role for pro-resolving lipids in the silencing of pain (Box 3) during the resolution phase of inflammation. Therefore, inflammatory lipids have a key role in the modulation of pain signaling.

Cytokines in Pain

Inflammatory cytokines derived from immune cells are critical mediators of nociceptor activity and pain sensitization. A historical perspective on the role of cytokines in pain has been provided elsewhere [26]. The first cytokine to be described as being hyperalgesic was IL-1β [27]. This was a seminal finding in the field of neuroimmunology that demonstrated that a molecule considered as part of the immune system could induce neuronal sensitization and that a receptor antagonist could inhibit its endogenous hyperalgesic effect [27]. Overtime, a role for cytokines in pain modulation has been demonstrated in almost all types of painful disease condition, including arthritis, neuropathic pain, and cancer-related pain [26]. In particular, IL-1β, IL-6, TNFα, IL-17A, and IL-5 have been shown to act directly on nociceptor neurons (Figure 1). IL-1β sensitizes

Box 3. Anti-inflammatory Lipids, Pro-resolving Lipids, and Pain Blockade

It has been found that certain anti-inflammatory and pro-resolving lipids have significant abilities to silence pain. Anti-inflammatory PGJ_2 signals to block pain by activating PPARγ and indirect activation of K^+_{ATP} channels in nociceptors [108]. Pro-resolving lipids include lipoxins, resolvins, protectins, and maresins, and have generally been shown to have analgesic effects [109,110]. For instance, spinal cord astrocytes express the lipoxin receptor ALXR/FPR2, and lipoxin A4 reduces inflammatory pain by inhibiting ERK and JNK activation in astrocytes. The Resolvin E1 (RvE1) receptor (ChemR23) is expressed by TRPV1-positive neurons. RvE1 inhibits TNFα and capsaicin-induced ERK activation in these DRG and spinal cord neurons. As a result, there is reduced release of excitatory glutamate, leading to diminished pain sensitivity [110]. Protectin D1 inhibits the neuronal plasticity induced by TRVP1 activation and TNFα [111], and maresin 1 inhibits TRPV1 currents in DRG neurons and inflammatory pain [110]. Neither Protectin D1 nor maresin 1 affect nociceptive mechanisms downstream of TRPA1 [110,111]. Taken together, these results are the flip side of the role of proinflammatory lipids, such as PGE_2 and LTB_4, in activation of pain. Therefore, lipid mediators have an important role in both inducing and silencing nociceptor neuron sensitization and activation. Future treatment of pain may involve the induction or administration of PGJ_2 and pro-resolving lipids.

nociceptor neurons via p38 MAPK phosphorylation of Nav1.8 sodium channels, leading to increased action potential generation and resulting in mechanical and thermal hyperalgesia [28]. IL-1β also activates IL-1R1 on nociceptor neurons to increase TRPV1 expression and, consequently, pain sensitivity to thermal stimuli [29]. IL-6 also contributes to inflammatory pain by inducing prostaglandin production [30] and by binding to its signal transducer gp130 expressed by nociceptors, leading to increased TRPV1 and TRPA1 expression [31,32]. TNFα-induced neuronal sensitization and hyperalgesia is dependent on TRPV1 and TRPA1 [33,34]. This sensitization is also linked to neuronal production of prostaglandins, because TNFα-induced capsaicin responsiveness in cultured nociceptors can be blocked with COX-2 inhibitors [35]. In agreement, TNFα-induced inflammatory pain *in vivo* is dependent on both TNFR1 and prostaglandins [30,36]. TNFα also induces rapid modulation of nociceptor sensitivity by p38MAPK-mediated phosphorylation of Nav1.8 and $Na_v1.9$ sodium channels to alter neuron excitability [33,37,38]. In a model of cancer-related pain, TNFα acted via TNFR2 to increase TRPV1 expression, resulting in thermal hyperalgesia [39]. Therefore, accumulating evidence shows that IL-1β, IL-6, and TNFα act via signaling mechanisms to induce prostaglandin synthesis and/or to potentiate TRP and Nav channel activation, leading to rapid sensitization of nociceptor neurons. Autoimmune diseases, such as arthritis and psoriasis, are painful. It is interesting to note that those diseases are associated with Th17 responses, and that IL-17A receptors are broadly expressed by nociceptor neurons. IL-17 induces a fast increase in neuronal excitability, suggesting a functional role of IL-17A in pain during autoimmune diseases [40]. In addition, IL-17A induces hyperalgesia dependent on amplification of TNFα, IL-1β, CXCL1, endothelin-1, and prostaglandins in antigen-induced arthritis [41]. Mast cells, Th2 cells, and ILC2s produce IL-5, a cytokine that mediates type 2 immunity. IL-5 can also sensitize nociceptor neurons expressing IL-5 receptors [42]. An interesting remaining question is whether specific subsets of T cells (e.g., Th1/17/2) induce distinct modalities of pain, whether heat, cold, or mechanical, due to their action on specific types of nociceptor neuron (see Outstanding Questions).

Immune-Derived Growth Factors and Neurotransmitters in Pain

Tissues that are highly innervated by nociceptor neurons are more responsive to noxious stimuli and this explains why different parts of the body present differential pain sensitivities. However, innervation is a dynamic process modulated by neurotrophic factors that are often upregulated during tissue injury and inflammation. These neurotrophic factors not only are important to restore the nerve density of an injured area, but also contribute to increased pain sensitivity. Nerve growth factor (NGF) is produced by innate immune cells during inflammation and activates its receptor, TrkA, in nociceptor neurons (Figure 1). Activation of TrkA activates PI3K/Src kinase signaling, which leads to phosphorylation of TRPV1 that, in turn, is rapidly inserted into the membrane, explaining the rapid sensitizing actions of NGF [43,44]. Further corroborating the NGF-induced translocation of TRPV1 to the membrane, NGF induces p38 MAPK activation in DRG neurons, increasing the membrane positivity to TRPV1 in peripheral terminals

independently of transcription [45]. Additionally, the neurotrophic activity of these molecules causes sprouting of axon terminals and contributes to increased local pain sensitivity and autotomy [46,47]. Neurotransmitters, including histamine and serotonin (5-HT), are also released by immune cells to modulate pain signaling. Mast cells contain histamine- and 5-HT-rich granules that are released upon activation. Histamine binds to H1 and H2 receptors in nociceptor neurons to increase the expression of $Na_v1.8$ channels and cause increased sensitivity to mechanical and thermal stimuli. A critical role for histamine in pain is not restricted to allergic inflammatory conditions, because it is also an important mediator of neuropathic pain induced by sciatic nerve ligation [48] and inflammatory pain induced by AITC, capsaicin, and formalin [49–51]. 5-HT binding to the 5-HT2 receptor and PKC activation increases the expression of neuronal acid-sensing ion channels (ASICs), which sense extracellular protons and mediate increased pain signaling [52].

Microglia Regulation of Central Pain Sensitization

Pain experience depends on the efficient transmission of nociceptive information from peripheral nociceptor neurons to second-order interneurons in the spinal cord. The spinal cord dorsal horn is the site where synapses between these neurons occur and it critically controls pain intensity [53]. It is increasingly clear that the action of immune cells and their mediators within the dorsal horn at both presynaptic sites (DRG neuron central terminals) and postsynaptic sites (interneurons) has an important role in pain sensitivity. While peripheral sensitization increases nociceptive inputs to the spinal cord, spinal events triggered by these inputs lead to 'central sensitization', a process critical for the persistence of pain, and contributes to its chronicity [54]. Upon nerve injury or in chronic pain conditions, nociceptor neurons express and release inflammatory mediators into the spinal cord, including neurotransmitters (e.g., CGRP), cytokines (e.g., CCL2, CX3CL1, and TNFα), growth factors (e.g., CSF-1), ATP, and enzymes (e.g., Caspase-6) via their central nerve terminals. These mediators communicate with and activate microglia, a key immune cell in central pain sensitization mechanisms [55–58] (Figure 2). Microglia are resident innate immune cells of the spinal cord and brain that act as sentinels of neuronal activity. They develop from a myeloid lineage and not only share similarities with peripheral macrophages, including the production of TNFα, IL-1β, and PGE_2, but also generate neurotrophins, including brain-derived neurotrophic factor (BDNF), that sensitize primary nociceptor neurons and second-order pain-mediating interneurons [59]. T cells also infiltrate the spinal cord during chronic pain, and have a role in neuronal sensitization (Figure 2). Curiously, the role of T cells and microglia in spinal sensitization may be gender specific. In male mice, p38 activation in microglia following peripheral inflammation induces their expression of BDNF, which activates and sensitizes postsynaptic neurons to induce mechanical hyperalgesia, while, in female mice, this process is primarily mediated by infiltrating T cells [60,61]. Microglia signaling also activates resident astrocytes and oligodendrocytes, two glial cell types that are sources of inflammatory mediators. Peripherally injured primary afferent nociceptor neurons release CX3CL1 into the spinal cord, which activates microglial production of TNFα in a p38 MAPK-dependent manner. In turn, TNFα activates spinal cord astrocytes to produce CCL2 in a JNK MAPK-dependent mechanism. CCL2 activates central neurons through CCR2, culminating in neuropathic pain [58]. Astrocytes also secrete CXCL1, which activates spinal cord dorsal horn neurons expressing CXCR2 in cancer pain models [62]. Recently, it was demonstrated that spinal cord oligodendrocytes contribute to neuropathic pain by producing IL-33, which is able to activate microglia and astrocytes in mice [63]. Therefore, crosstalk between T cells, microglia, astrocytes, and oligodendrocytes in the spinal cord mediate central sensitization of neuronal circuits to produce chronic pain.

Nociceptor Neuron Regulation of Inflammation and Immunity

Emerging research is showing that nociceptor neurons have an active and significant role in regulating the immune response and inflammation. Thus, pain is not only a symptom of

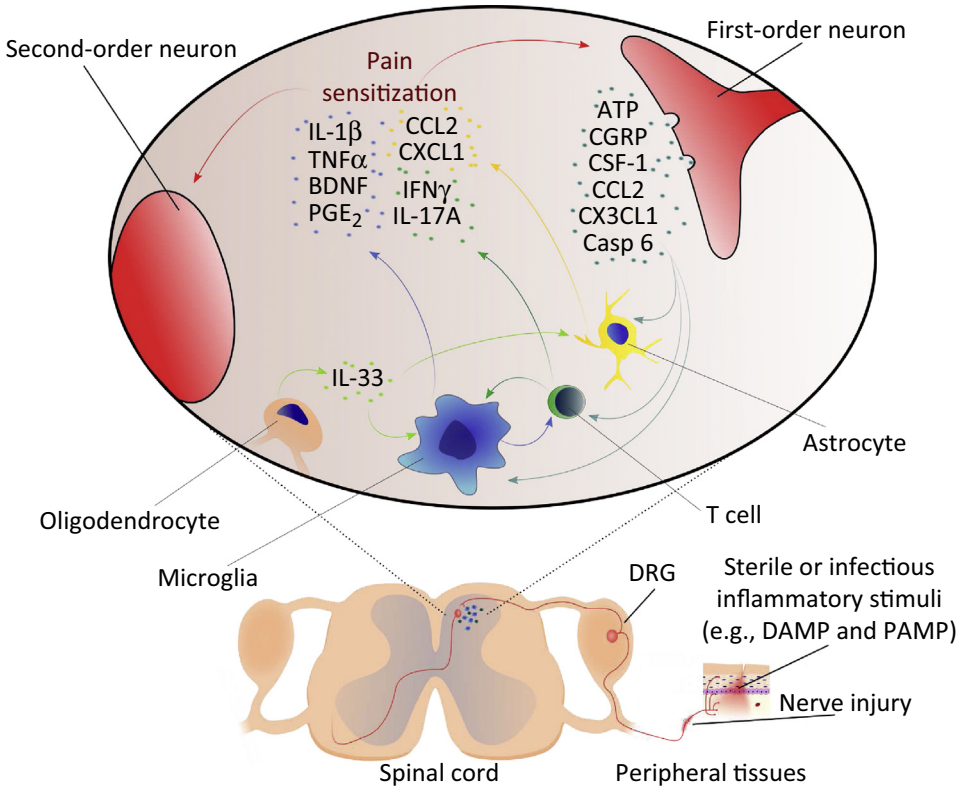

Figure 2. Microglia and T Cells Mediate Central Sensitization of Pain in the Spinal Cord. Microglia are the resident immune cells of the central nervous system, and have a key role in mediating central pain sensitization. Primary afferent nociceptor neurons transduce action potentials from the periphery to the dorsal horn of spinal cord, where synapses between first-order and second-order neurons occur. In chronic inflammatory or neuropathic pain, nociceptors release mediators, including caspase 6 (Casp 6), ATP, chemokine ligand 2 (CCL2), tumor necrosis factor alpha (TNFα), colony-stimulating factor-1 (CSF-1), and calcitonin gene-related peptide (CGRP), that activate microglia. Microglia produce inflammatory mediators, including interleukin 1 beta (IL-1β), TNFα, brain-derived neurotropic factor (BDNF), and prostaglandin E_2 (PGE_2), which sensitize first- and second-order neurons. This process is called 'spinal sensitization' and contributes to chronic pain. T cells also infiltrate the spinal cord and cross-talk with microglia cells and neurons to amplify pain sensitivity. Upon peripheral nerve injury, primary afferent nociceptor neurons release CX3CL1 into the spinal cord, which induces dorsal horn microglia to produce TNFα, which activates astrocytes to produce CCL2 and CXCL1, which induce changes in spinal cord neurons leading to central sensitization. Oligodendrocytes produce IL-33 and cross-talk with microglia and astrocytes to increase pain sensitivity. Abbreviations: DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern.

inflammation, but also an active participant in regulating immunity. Upon activation by noxious and/or harmful stimuli, nociceptors release neuropeptides and neurotransmitters from their peripheral terminals that have potent effects on the vasculature and on the function of innate and adaptive immune cells (Figure 3). Dendritic cells, neutrophils, macrophages, mast cells, and T cells express receptors for these neuronal mediators, allowing them to respond directly to nociceptors. Nociceptors also participate in neural reflex circuits through other neuronal subtypes that dampen inflammation (reviewed in [64]). Here, we highlight how nociceptor neurons have an active role in modulating vascular function, immune cells function, and inflammation in health and disease conditions (Figure 4).

Neuronal Regulation of Vasculature and Lymphatic Vessels

Neuronal regulation of inflammation was first demonstrated by experiments showing that chemical irritants produced redness, heat, and swelling dependent on sensory nerve supply

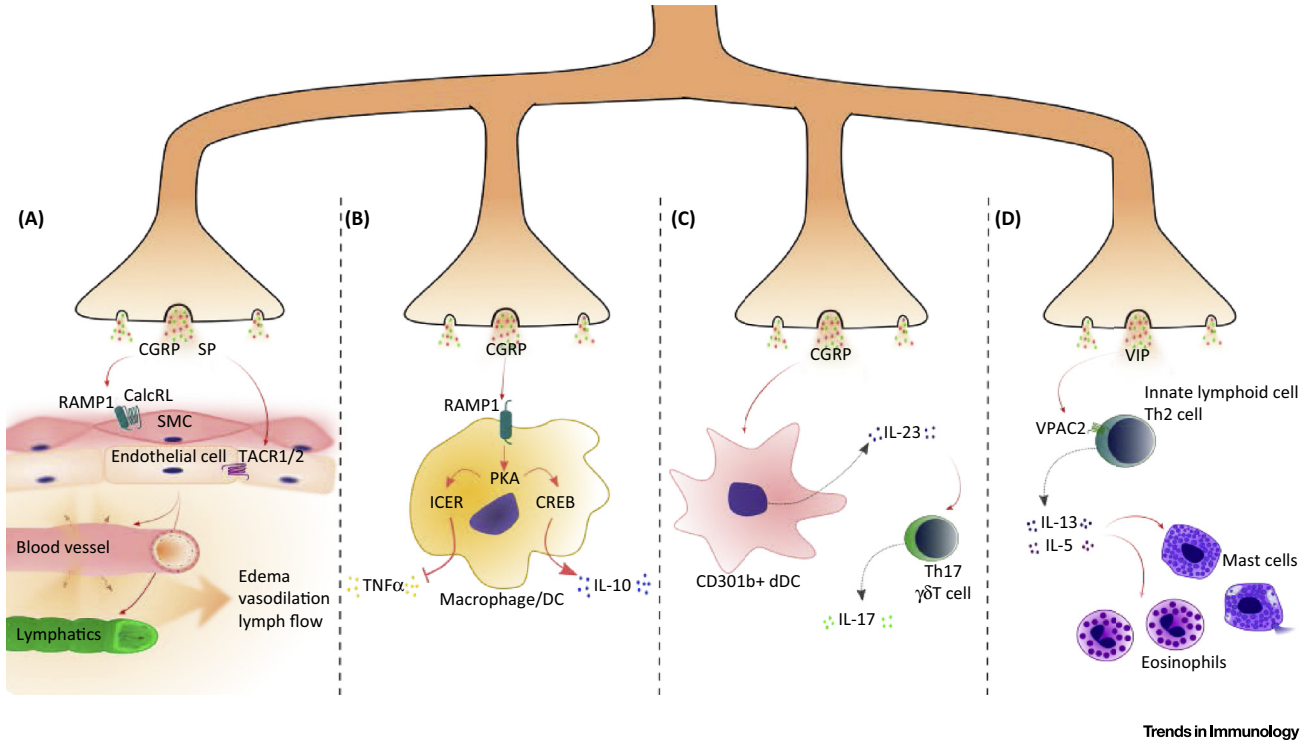

Figure 3. Nociceptor Neurons Release Neuropeptides that Regulate Vascular, Innate, and Adaptive Immune Cell Function. While noxious stimuli generate pain through afferent signals to the central nervous system, calcium influx in the peripheral nerve terminals also causes local release of dense-core vesicles containing neuropeptides. These neuropeptides have potent effects on the vasculature and immune cells to regulate tissue inflammation: (A) The neuropeptide calcitonin gene-related peptide (CGRP) activates the RAMP1/CalcRL receptor complex in vascular smooth muscle cells (SMC) to promote muscle relaxation and vasodilation. Substance P (SP), another nociceptive neuropeptide, activates tachykinin receptor 1 and 2 (TACR1/2) in vascular endothelial cells to increase vascular permeability, which results in edema formation. CGRP and SP also act on lymphatic endothelial cells and SMC to regulate lymph flow. (B) CGRP binds RAMP1 in macrophages and dendritic cells (DC), leading to downstream PKA activity, which affects cytokine production by two different pathways. The first pathway (left side) occurs by induction of the transcriptional inducible cAMP early repressor (ICER) and inhibition of tumor necrosis factor alpha (TNFα) expression. The second pathway (right side) occurs by induction of the transcription factor cAMP response element binding (CREB) and induction of interleukin 10 (IL-10) expression. (C) CGRP increases IL-23 production by dermal dendritic cells (CD301b+ dDCs) that, in turn, promotes IL-17 production by Th17 cells and γδT cells. (D) Vasoactive intestinal peptide (VIP) activates its receptor VPAC2 expressed by innate lymphoid cells and Th2 cells and stimulates these cells to produce IL-5 and IL-13, important mediators of allergic reactions that cause the degranulation of eosinophils and mast cells.

[65]. Electrical nerve stimulation directly produced vasodilation and permeability, a process termed 'neurogenic inflammation' [66]. This process is mediated by axon–axon reflexes, whereby calcium influx and antidromic signaling (back-propagation of action potentials) leads to signal transduction in neighboring axons and the release of mediators stored within dense-core vesicles located at the axon terminals in the periphery. Nociceptor mediators are diverse (Box 4), including the neuropeptides calcitonin-gene related peptide (CGRP) and substance P (SP), which are some of the most potent mediators of vasodilation and tissue edema known (Figure 3A) [67]. They act directly on smooth muscle cells and vascular endothelial cells to mediate neurogenic inflammation. Nociceptor nerve fibers are also closely associated with lymphatic vessels. SP stimulates lymphatic vessel contractility and increases pump efficiency through its receptors TACR1 and TACR3, expressed by lymphatic smooth muscle cells [68,69]. CGRP increases the constriction frequency of perfused lymphatic vessels, a phenomenon that is dependent on nitric oxide [70]. The CGRP receptor RAMP1 is necessary for proper angiogenesis and lymphangiogenesis during skin wound healing, and regulates VEGF production [71]. Despite this evidence for interactions between nociceptor neurons with blood and lymph vessels, it remains to be determined whether this crosstalk shapes antigen drainage and adaptive immunity. An interesting question would be to determine whether nociceptor neurons regulate the initiation or priming of T or B cell responses in draining lymph nodes.

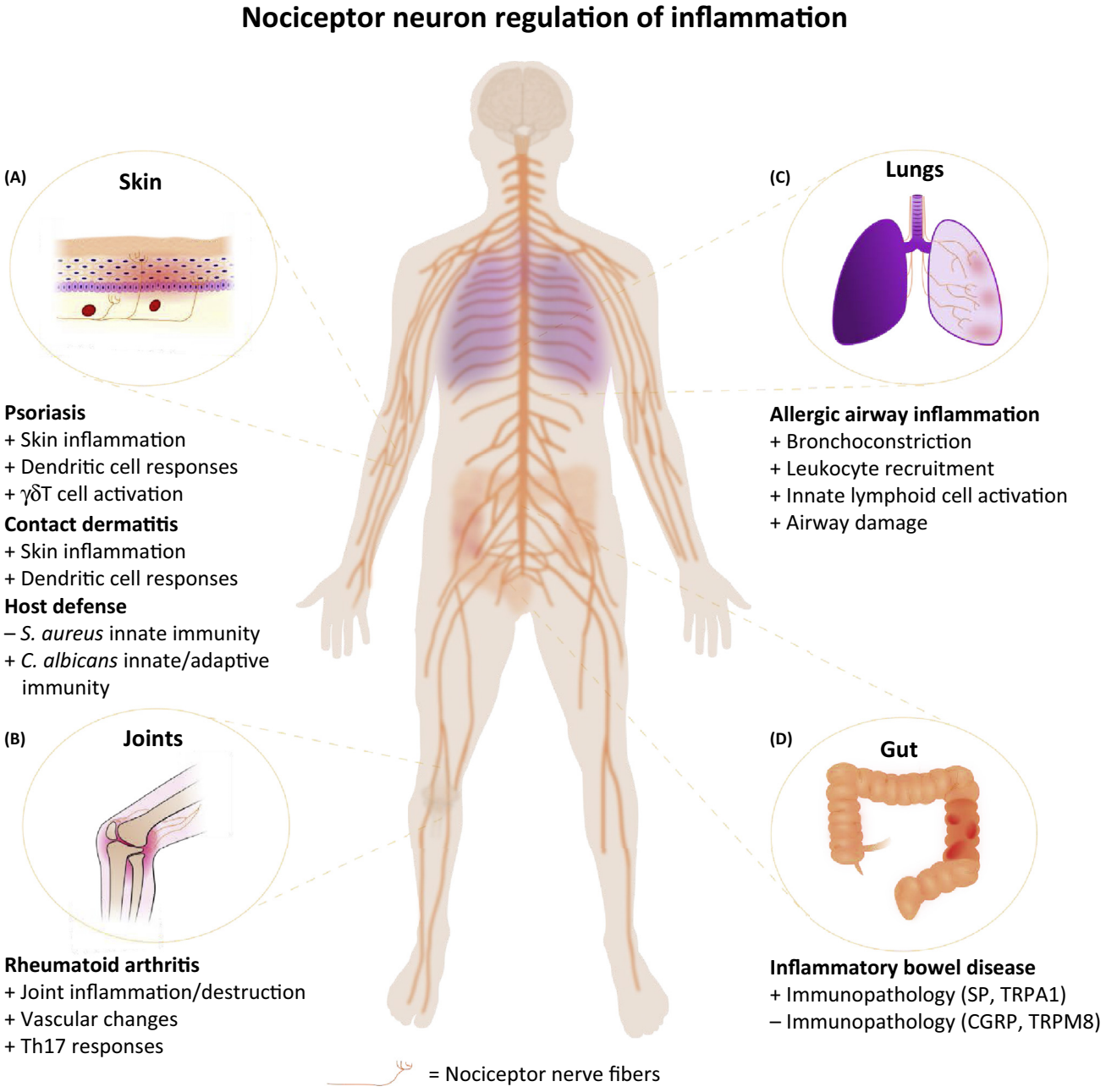

Figure 4. Nociceptor Neurons Actively Contribute to Inflammatory Disease Conditions. Nociceptor neurons actively modulate the immune response and disease progression in inflammatory conditions. (A) In the skin, nociceptor neurons have a role in driving dendritic cell activation and γδT cell IL-17 production in psoriasis-like inflammation. They also have a role in mediating oxazalone and FITC-driven mouse models of contact dermatitis. Nociceptor neurons reduce skin host protection against *Staphylococcus aureus* infection, but promote skin immunity against *Candida albicans* (B) In joints, neurons regulate the severity of rheumatoid arthritis due to their effects in promoting Th17 cell responses and changes in vascular endothelial cells. (C) In the lungs, nociceptor neurons contribute to asthmatic airway inflammation and its deleterious effects by driving type 2 innate lymphoid cells and mediating bronchoconstriction. (D) In the gastrointestinal tract, nociceptor neurons regulate the progression of mouse models of colitis. While nociceptor neurons drive immunopathology (cytokine production and weight loss) through mechanisms related to substance P (SP) release and activation of the nociceptive ion channel TRPA1, they also reduce immunopathology through release of calcitonin gene-related peptide (CGRP) and activation of the cold-sensing ion channel TRPM8.

Box 4. Neuropeptides and a Role in Migraine

Nociceptive neurons express a diverse set of neuropeptides, which are stored in dense core granules and released both at central and peripheral terminals during activation. Some well-studied nociceptive neuropeptides include calcitonin-gene related peptide (CGRP), substance P (SP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), Galanin (Gal), and Somatostatin (SST). These neuropeptides have distinct expression patterns within nociceptive neurons, and also bind to different G-protein-coupled receptors on their target tissues. Neuropeptides also mark distinct innervation patterns by nociceptors. For example, in the skin, CGRP+ 'peptidergic' nociceptors innervate the stratum spinosum of the epidermis and the dermal–epidermal interface, whereas Mrgprd+ neurons innervate the superficial stratum granulosum layer [112]. Therefore, neuropeptide release could be a key mechanism by which nociceptors regulate central nervous system and peripheral responses.

CGRP, in particular, also has a critical role in migraine. Migraine is a neurological disorder with complex mechanisms involving alterations of sensory perception and processing. A common feature of most migraine headaches is the involvement of the trigeminovascular structure, which encompasses an anatomical unit including cortex, hypothalamus, brainstem, trigeminal nerve, and meninges. The release of neuropeptides such as CGRP may be an underlying basis of increased pressure and pain of migraine. In fact, CGRP is a promising target in clinical trials to treat the chronic pain of migraine. Initial use of small molecules targeting CGRP in migraine has shown promise, although has adverse effects. However, these adverse effects did not occur following the use of recent antibody blockade strategies developed to target CGRP. CGRP levels are elevated in spontaneous or induced experimental migraine; CGRP infusion induces migraine, and targeting CGRP in Phase II and III trials reduced migraine symptoms, including photophobia. Mechanistically, it is thought that trigeminal afferent nerve fibers release CGRP in the dura mater and subarachnoid space during migraine, which then binds to and activates RAMP1, causing vasodilatation and induces dura mater mast cell degranulation and neuroinflammation. As a consequence, CGRP induces peripheral and central sensitization observed as pain, photophobia, and phonophobia. The animal data [113], advances in targeting CGRP in migraine [114], and migraine mechanisms and CGRP systemic functions [115] have been reviewed elsewhere.

Neuronal Regulation of Host–Pathogen Defense

Bacterial, viral, and fungal infections are often accompanied by pain. Recent studies showed that nociceptor neurons actively participate in mammalian host responses against pathogens. Nociceptor neurons are able to directly sense bacteria and fungal-derived molecules, including lipopolysaccharide (LPS), flagellin, bacterial toxins, and zymosan, to produce pain during infection (reviewed in [72,73]). Nociceptor neurons then release mediators that modulate the function of macrophages, dendritic cells, T cells, and innate lymphoid cells. The effects on the outcome of host defense can be protective or harmful, depending on the type of pathogenic infection.

Nociceptor neurons drive protective skin immunity against the fungal pathogen *Candida albicans* [74]. In a mouse model of *C. albicans* infection, nociceptor neuron deficiency led to significantly reduced IL-23 production by CD301b+ dermal dendritic cells, which was required for IL-17A production by skin γδT cells. The result was significantly reduced host clearance of *C. albicans*. These defenses were restored by repeated injections of CGRP during infection in nociceptor-deficient mice. *Candida albicans* and its cell wall component zymosan activate nociceptor neuron calcium flux [74]. Therefore, nociceptor neurons mediate dendritic cell and T cell responses against cutaneous *C. albicans* invasion.

By contrast, nociceptor neurons downmodulate immune responses against the Gram-positive bacterium *Staphylococcus aureus*. In *S. aureus* skin infection, pain is produced by neuronal sensing of bacterial *N*-formylated peptides and the pore-forming toxin α-hemolysin [75]. Nociceptor neuron deficiency led to increased neutrophil and monocyte infiltration at the infection site and draining lymph node hypertrophy. The nociceptor-derived neuropeptides CGRP, galanin, and somatostatin decreased macrophage TNFα production in response to *S. aureus*, and CGRP administration restored lymph node hypertrophy, including the recruitment of T cells and B cells during infection, although the effects on subsequent adaptive immunity have not yet been determined [75]. CGRP potently upregulates IL-10 and downregulates TNFα expression in macrophages and dendritic cells via two pathways: CGRP induces IL-10 through PKA-dependent activation of cAMP response element binding (CREB), while TNFα inhibition is

mediated by PKA-dependent signaling through inducible cAMP early repressor (ICER), which silences ATF-2 binding to the TNFα promoter [76–78] (Figure 3B).

While nociceptor suppression of innate immunity may not be beneficial during localized bacterial infections, it may be important in suppressing systemic immunopathology during bacterial sepsis. In LPS-induced shock, the nociceptor-derived neuropeptides CGRP, vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP) decreased cytokine levels and mortality in mice with exogenous administration [79,80]. In a model of polymicrobial septic peritonitis, deficiency in RAMP1 led to increased innate immune cell recruitment and decreased IL-10 levels [77]. Thus, nociceptor neuron-derived neuropeptides limit the innate inflammatory response. In the cecal ligation and puncture (CLP) model of sepsis, absence of TRPV1 led to increased cytokine levels, decreased macrophage phagocytosis, and bacterial clearance, resulting in an exacerbated inflammatory response [81]. LPS potently induces inflammatory pain, a mechanism dependent on TLR4/MyD88 but not on TLR4/TRIF signaling [82]. LPS also directly activates nociceptor neurons by gating the nociceptive ion channel TRPA1, a process mediated by the Lipid A moiety [83].

In addition to a role in sepsis, the nervous system also regulates gastrointestinal defenses against bacterial pathogens. Blockade of TACR1 led to increased IgA and Th2 cytokine production, a mechanism that is protective against *Salmonella enterica* infection [84]. Gut extrinsic sympathetic neurons are activated downstream of *Salmonella* infections in the gut lamina propria and trigger a site-restricted anti-inflammatory polarization in macrophages in the myenteric plexus without affecting the response of macrophages in superficial layers [85]. A better understanding of the molecular interactions between nociceptor neurons, pathogens, and immune cells may lead to novel approaches to treating infectious diseases.

Neuronal Regulation of Inflammatory Diseases

Pain is characteristic of many chronic inflammatory diseases. Recent work has shown that nociceptors actively regulate joint, skin, lung, and gastrointestinal diseases, and targeting pain could treat inflammation (Figure 4).

Nociceptor neurons drive inflammation in psoriasis and contact dermatitis. It was found that chemical denervation of TRPV1+ nociceptors or genetic ablation of $Na_v1.8$+ neurons led to decreased skin pathology in the Imiquimod-based mouse model of psoriasis [86]. This study found that nociceptors drive dendritic cell production of IL-23, which mediates γδT cell expression of IL-17 (Figure 3C) [86]. Nociceptor neurons also directly respond to the haptens oxazolone and urushiol, the contact allergen of poison ivy, a response dependent on the ion channel TRPA1 [87]. $TRPA1^{-/-}$mice showed significantly less inflammation in acute and chronic models of oxazolone-contact dermatitis [87]. CGRP and its receptor RAMP1 are multifaceted, having an inhibitory role in Th1-driven 2,4,6-trinitrochlorobenzene (TCNB) contact dermatitis, while promoting Th2-driven fluorescein isothiocyanate (FITC) contact dermatitis [88]. CGRP activates RAMP1 to inhibit dendritic cell production of IL-12, IL-6, and TNFα and expression of CCR7, leading to downregulation of Th1 cell differentiation [88,89]. The inhibitory effects of CGRP on macrophages differ from those on dendritic cells, in that IL-10-producing macrophages still migrate to the lymph node, where they contribute to antigen presentation and induction of Th2 responses [88]. Therefore, nociceptor neurons drive or suppress inflammation depending on crosstalk with specific innate and adaptive cell types (Figure 4A).

Rheumatoid arthritis is characterized by joint swelling and intense pain. Interestingly, denervation decreases joint pathology in both human and animal models, a process that may be due to nerve–vascular interactions. In the K/BxN model of serum-transfer arthritis, it was found that denervation led to significant changes in the transcriptome of vascular endothelial cells [90].

CGRP stimulates endothelial cells to produce IL-6, which generates Th17 cells following antigen presentation and, consequently, IL-17A production [91], a process that could contribute to autoimmune pathology. In mBSA antigen-induced arthritis (AIA), the initial steps of joint inflammation are abrogated when the IL-6 signal transducer is knocked out of $Na_v1.8$-positive nociceptor neurons [92]. By contrast, removal of TRPV1-positive nociceptor neurons by pretreatment with resiniferatoxin (RTX) exacerbates joint inflammation in the K/BxN arthritis model [93] (Figure 4b). This could be due to differences in animal models as well as the role of specific nociceptor subtypes targeted in these studies.

Nociceptors may also have a critical role in driving lung hyperreactivity and airway inflammation in asthma [42,94,95] (Figure 4C). The respiratory tract is innervated by nociceptor neurons that can induce cough, mucus production, and bronchoconstriction. These neurons detect noxious stimuli, including chemical irritants and allergens. Blockade of the nociceptive ion channel TRPA1 or treatment of mice with the TRPA1 antagonist HC-030031 led to reduced allergic airway inflammation and immune cell recruitment in a mouse model of asthma [94]. Targeted ablation of TRPV1+ nociceptors or Tetanus toxin-mediated silencing of these neurons led to decreased bronchoconstriction [95]. Silencing of nociceptor neuron activity using a charged form of lidocaine (QX-314) and targeted ablation of $Na_v1.8$-lineage neurons led to reduced allergic airway inflammation and immune cell influx [42]. Nociceptor neurons may drive lung inflammation through their release of the neuropeptide VIP, which acts on the VPAC2 receptor expressed by Type 2 innate lymphoid cells (ILC2) to induce IL-5 production [42] (Figure 3D). Neuropeptides also potentiate degranulation of mast cells, key mediators of allergic inflammation [5,96]. Thus, silencing neurons may be a strategy to treat asthmatic lung inflammation.

Nociceptor neurons actively regulate inflammation in gastrointestinal diseases (Figure 4D). Pain affects the quality of life of patients with irritable bowel syndrome (IBS), Crohn's disease, and ulcerative colitis. In dextran-sulfate-sodium (DSS) and 2,4,6-trinitrobenzene-sulfonic-acid (TNBS) models of colitis, the nociceptive ion channel TRPA1 is activated, resulting in neuronal SP release, which drives colonic inflammation [97]. By contrast, TRPM8, a nociceptive ion channel mediating cold sensation, attenuates cytokine levels and immunopathology in DSS and TNBS colitis models [98]. TRPM8+ mucosal sensory neurons release CGRP, which suppress colitogenic myeloid cell signaling [99]. Therefore, nociceptor neurons differentially regulate colonic inflammation depending on the type of nociceptive ion channel or neuropeptide involved.

In addition to direct communication with immune cells, nociceptor neurons also have an active dialog with epithelial cells during inflammation. CGRP activates MAPK pathways to induce keratinocyte proliferation and production of TNFα, IL-1β, IL-6, and NGF [100]. CGRP and SP induce NLRP1/caspase-1 inflammasome signaling in keratinocytes, which mediates IL-1β-dependent mechanical hyperalgesia [101]. In addition to the classic free nerve endings of nociceptor neurons in peripheral tissues, nociceptors innervate specialized structures, where they have neuro-endocrine roles that can be immunomodulatory. In the lungs, pulmonary neuroendocrine cells (PNECs) are sensory organoids comprising innervated epithelial cells that produce CGRP. Dysfunctional PNECs result in increased CGRP release and immune cell recruitment in lung disease [102]. Future work will determine and define the importance of specific nociceptor neuron–immune and neuron–endocrine interactions in different inflammatory disease conditions.

Concluding Remarks

It is increasingly clear that nociceptor neuron–immune interactions have a critical role in pain and inflammation. The immune and nociceptive systems are specialized to recognize damaging and/or harmful stimuli, and their functional interactions may have important roles in driving responses to prevent tissue damage and restore homeostasis. Dysregulation of these interactions could

Outstanding Questions

How do nociceptor neurons intersect with other branches of the nervous system (autonomic and enteric) during the regulation of peripheral immune responses?

Do distinct innate and adaptive immune cell types communicate with nociceptor neuron subsets as determined by their phenotypic or anatomical categorization?

Do certain immune mediators, cytokines, or lipids potentiate specific pain modalities? For example, is there an 'immunological code' for heat, cold, or mechanical pain? Is there a similar 'immunological code' for nociceptor neuron neuropeptide release?

How do immune mediators regulate epigenetic and transcriptional changes in nociceptor neurons to make the transition from acute to chronic pain?

Do nociceptor neurons regulate the immune response during the transition of acute inflammation to its resolution phase?

How do neurotransmitters and neuropeptides signal to change the functional phenotypes of innate and adaptive immune cells?

What role do nociceptor neurons have in modulating immune responses to cancer?

Do central nervous system circuits activated by pain signaling have a role in regulating peripheral inflammation through neuroendocrine or autonomic reflexes?

Does pain blockade by current analgesic approaches (e.g., opioids) lead to defects in host–pathogen defense or immune-mediated disease outcomes?

Can blockade of pain signaling produce new treatments for chronic inflammatory diseases, including rheumatoid arthritis, psoriasis, asthma, and colitis?

underlie the pathogenesis of inflammatory diseases in the skin, joint, respiratory, and gastrointestinal tracts. These neuroimmune interactions occur within peripheral sites of injury, as well as in the central nervous system. Targeting specific immune cells, cytokines, or lipid mediators may lead to novel approaches to treat chronic pain. Conversely, modulation of nociceptor neuron activity or mediators could lead to new approaches to treat infection and chronic inflammatory diseases. Altogether, it is clear that the sensory nervous system is a key modulator of host protective responses and understanding its interactions with immune cells could reveal new mechanisms that could be targeted to treat and prevent diseases.

Acknowledgments

F.A.P-R. acknowledges the PhD fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil). W.A.V.J. acknowledges support from the Center for Research in Inflammatory Disease (CRID, Brazil), and Senior Researcher fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil). I.M.C acknowledges funding and support from NIH/NCCIH DP2AT009499, NIH/NIAID K22AI114810, and the Harvard Digestive Disease Center, NIH grant P30 DK348345.

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